AWARD NUMBER: W81XWH-13-2-0060

TITLE: "The Johns Hopkins RTR Consortium: A Collaborative Approach to Advance Translational Science and Standardize Clinical Monitoring of Restorative Transplantation – Immunomodulation and Tolerance Induction after VCA using Biologic Agent (cTLA4-Ig) and Donor Bone Marrow Cells"

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REPORT DATE: October 2014

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2014	Annual	15 Sep 2013 - 14 Sep 2014

4. TITLE AND SUBTITLE
"The Johns Hopkins RTR Consortium: A Collaborative Approach to Advance Translational Science and Standardize Clinical Monitoring of Restorative Transplantation – Immunomodulation and Tolerance Induction after VCA using Biologic Agent (cTLA4-Ig) and Donor Bone Marrow Cells"

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-13-2-0060

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT For many devastating combat and civilian injuries where conventional reconstruction is not achievable, vascularized composite allotransplantation (VCA) has become a viable alternative. However, the toxicities and adverse effects of high dose immunosuppressive drugs have curtailed wider application. Thus the purpose of this project is to develop novel clinically relevant regimens for immunomodulation and tolerance induction after VCA using a translational large animal model.During the current reporting period the group set out to establish a belatacept-based protocol to enable calcineurin inhibitor minimization after heterotopic swine hind-limb allotransplantation across a full SLA mismatch (Aim 1). All animals were induced with 100 cGy whole body irradiation and 700 cGy thymic irradiation. Group I animals received high dose tacrolimus postoperatively (10-20 ng/dl), Group II animals received low dose tacrolimus postperatively (4-6 ng/dl), and Group III animals will receive low dose tacrolimus with intermittent belatacept (CTLA4-Ig). All Group I animals (n=3) have undergone transplantation and have shown no signs of rejection. However, 2 of 3 animals died prematurely (POD 70 and 96) due to infectious complications related to high dose tacrolimus. One animal from Group II has been completed and underwent rejection within 20 days of lowering the tacrolimus dose into the target range of 4-6 ng/dl. Remaining Group II and all of Group III transplantations have been delayed due to unexpected irradiation issues. A total of four animals died prematurely (all within 15 days postoperatively) due to pancytopenia related to dosing complications resulting from technical problems with preoperative irradiation. Extensive discussions with the radiation oncology team, including further computed tomography scans of naïve pigs for precise irradiation planning, as well as dose validation studies have alleviated concerns of irradiation dosing issues for future transplants to complete Aim1. Overall the obtained results confirm Aim 1 hypotheses. High dose tacrolimus maintains allografts, but results in complications due to high dose immunosuppression. Low dose tacrolimus is not sufficient to maintain allografts alone, but does not result in adverse effects. Experiments to further investigate if the addition of belatacept will be sufficient to maintain allografts with low dose tacrolimus are currently ongoing.

15. SUBJECT TERMS
Vascularized composite allotransplantation (VCA), Hand transplantation, Face transplantation, Tolerance induction, Immunomodulation, Chimerism, Costimulatory blockade, Belatacept, CTLA4-Ig, Large animal model

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	19	19b. TELEPHONE NUMBER *(include area code)*

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1. **INTRODUCTION**

Close to 40% of combat injuries sustained in Operation Enduring Freedom (OEF) and Operation Iraqi Freedom (OIF) involved severe extremity and craniofacial trauma. Currently, despite the best reconstructive efforts using native tissue, these injuries are not only mutilating, but frequently result in permanent disfigurement and morbidity. For many devastating combat and civilian injuries where conventional reconstruction is not achievable, vascularized composite allotransplantation (VCA) has become a viable alternative. However, the toxicities and adverse effects of the high dose immunosuppressive drugs have curtailed wider application. In particular, the use of calcineurin inhibitors (CNIs, i.e. tacrolimus), currently the mainstay therapy in VCA, is associated with substantial morbidity and is relatively ineffective in preventing antibody-mediated injury and chronic rejection.

Thus, the central challenge for VCA is to develop novel treatment concepts to minimize/avoid immunosuppression and extend the benefits of these life-enhancing procedures to the military and civilian patient populations. Biologic agents such as the monoclonal antibody Cytotoxic T-lymphocyte-associated antigen-4 immunoglobulin (CTLA4-Ig) (e.g., abatacept, belatacept), which block T-cell costimulation, have been developed to overcome this limitation, and represent a new paradigm in immunosuppression - biological therapy for maintenance immunosuppression devoid of the toxicities associated with CNIs.

In this study we propose to use the second-generation, FDA approved, CTLA4-Ig belatacept, that has demonstrated potent inhibition of T-cell activation and proven effective in phase II and III trials of kidney transplantation, to develop clinically relevant regimens for immunomodulation and tolerance induction after VCA using a translational large animal model.

2. **KEYWORDS**

Vascularized composite allotransplantation (VCA)
Hand transplantation
Face transplantation
Tolerance induction
Immunomodulation
Chimerism
Costimulatory blockade
Belatacept
CTLA4-Ig
Large animal model

3. **ACCOMPLISHMENTS**

The group obtained approval for the proposed project from both the Institutional Animal Care and Use Committee (IACUC) at Johns Hopkins University as well as the Animal Care and Use Review Office (ACURO) of the Department of Defense.

During year one of this project a non-myeoloablative induction regimen was successfully established consisting of 100cGy total body irradiation (TBI) plus 700cGy thymic irradiation. This treatment protocol was subsequently employed *in vivo* in an immunologically stringent (full class I and class II SLA mismatch) swine heterotopic hind limb allotransplantation model. We have demonstrated that combining this induction regimen with high dose tacrolimus (10-20 ng/mL) maintenance therapy postoperatively successfully prevents rejection. However, as hypothesized, high dose tacrolimus treatment results in clinical complications; one of the primary motivations for this study. Furthermore, we have shown that low dose tacrolimus (4-6 ng/mL) monotherapy postoperatively is not adequate for preventing rejection. These preliminary results again represent a need for adjunct agents such as costimulatory blockade with belatacept to prevent rejection in the setting of post transplant immunosuppression (CNI) minimization.

a. **What were the major goals of the project?**

As outlined in the approved Statement of Work (SOW) for this project, the Year 1 Major Goals were as follows:

<u>Phase 1, Aim 1</u>: Establish a belatacept-based protocol to enable *CNI minimization* after VCA.

Experimental groups for this aim are summarized in Table 1 with current status (percentage of animals completed in each group). Recipient animals underwent induction therapy by means of low-dose total body (100 cGy) and thymic (700 cGy) irradiation. The temporal recovery and degree of immune cell depletion with this approach are both

similar to that seen with alemtuzumab used in our clinical immunomodulatory protocol for upper extremity transplantation. Delays in progress and incomplete groups will be discussed in detail in Section 5 – Changes/Problems.

Table 1. Aim 1 Experimental Groups

Group	N	SLA Mismatch	Protocol	Rational	Status
I	3	Full	Induction + high-dose TAC maintenance	Control Group: therapeutic CNI	100%
II	3	Full	Induction + low-dose TAC maintenance	Control Group: sub-therapeutic CNI	33%
III	5	Full	Induction + low-dose TAC + CTLA4-Ig maintenance	Experimental Group: Tests role of CTLA4-Ig to allow for graft survival with minimal CNI	0%

Animals in Group I will received high-dose tacrolimus maintenance therapy commenced on day 0 (target trough level 10-20 ng/ml) to mimic the current clinical standard CNI regimen. This group was anticipated to have long-term graft acceptance without clinical signs of rejection as long as therapeutic trough levels are maintained, and is used as baseline controls to assess the side effects and toxicity profile of CNIs in this model. Group II will control for the effects of sub-therapeutic, low-dose tacrolimus treatment (target trough level 4-6 ng/ml) defining the pace and degree of progressive acute rejection. In Group III recipient animals receive low-dose tacrolimus (target trough level 4-6 ng/ml) in combination with belatacept (20 mg/kg) given as intravenous infusion on Pod 2, 7, 14, 30, 60, 90, 120, 150.

The group proposed to perform protocol skin biopsies in all animals at day 15, 30, 60, 120 and 150 and to be assessed by H&E histology and immunohistochemistry for signs of rejection (CD3, CD4, CD8, CD20) or the presence of intragraft regulatory cells (Treg, Foxp3). Blood samples drawn from recipient animals at the same time points as protocol biopsies have been used for metabolic monitoring (CBC, LFT, CR, BUN, Glucose), the presence of donor specific antibodies (DSA) and FACS analysis of immune cell phenotypes. To assess CNI nephrotoxicity renal histology will be performed at study endpoint at POD 150.

b. **What was accomplished under these goals?**

<u>Phase 1, Aim 1, Year 1:</u> Establish a belatacept-based protocol to enable *CNI minimization* after VCA.

1.1 Aim 1, Tasks 1: Obtain institutional Animal Care and Use Committee (ACUC) approval

The major activity under Task 1 was to establish an IACUC approved animal research protocol. The specific objectives of this task were met by the approval of the IACUC

protocols, allowing the VCA Laboratory to perform the proposed *in-vivo* transplantation experiments.

1.2 Aim 1, Task 2: Obtain DoD Animal Care and Use Review Office (ACURO) approval

The major activity under Task 2 was to establish an ACURO approved animal research protocol. The specific objectives of this task were met by the approval of the ACURO protocols, allowing the VCA Laboratory to perform the proposed *in-vivo* transplantation experiments.

1.3 Aim 1, Task 3: Adapt clinically established induction and CNI maintenance regimen in a fully SLA-mismatched swine hind limb transplantation model

1.3.1 Aim 1, Task 3, Subtask 1: Perform hind limb transplantation with high-dose TAC maintenance therapy (Group I; n=3)

All animals in Group 1 (n=3) have undergone heterotopic hind limb allotransplantation. As hypothesized, high dose tacrolimus (trough level 10-20 ng/mL) has maintained all allografts with no clinical signs of rejection throughout the study period (Figure 1), demonstrated in clinical photographs of the graft skin component and histological sections in Figure 2. Tacrolimus trough levels and clinical laboratory results are shown in Figure 3 for the three animals from Group 1. In attempting to maintain high dose tacroliums, it was not uncommon for levels to reach significantly higher trough levels (i.e. 40-60 ng/mL) that were corrected, as is seen clinically when medication trough windows are set in a high range. Platelet levels were seen to drop significantly following irradiation, and would then recover around postoperative day 9-11 (post-irradiation day 11-13).

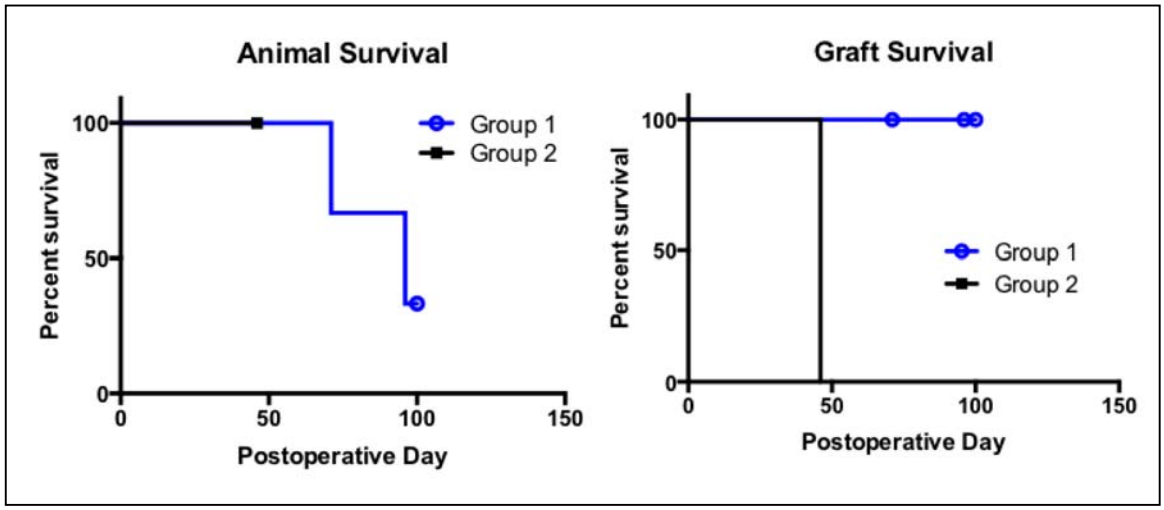

Figure 1. Animal and graft survival curves demonstrating maintained grafts in Group 1 with premature deaths, and rejection of graft in Group 2 without unexpected death.

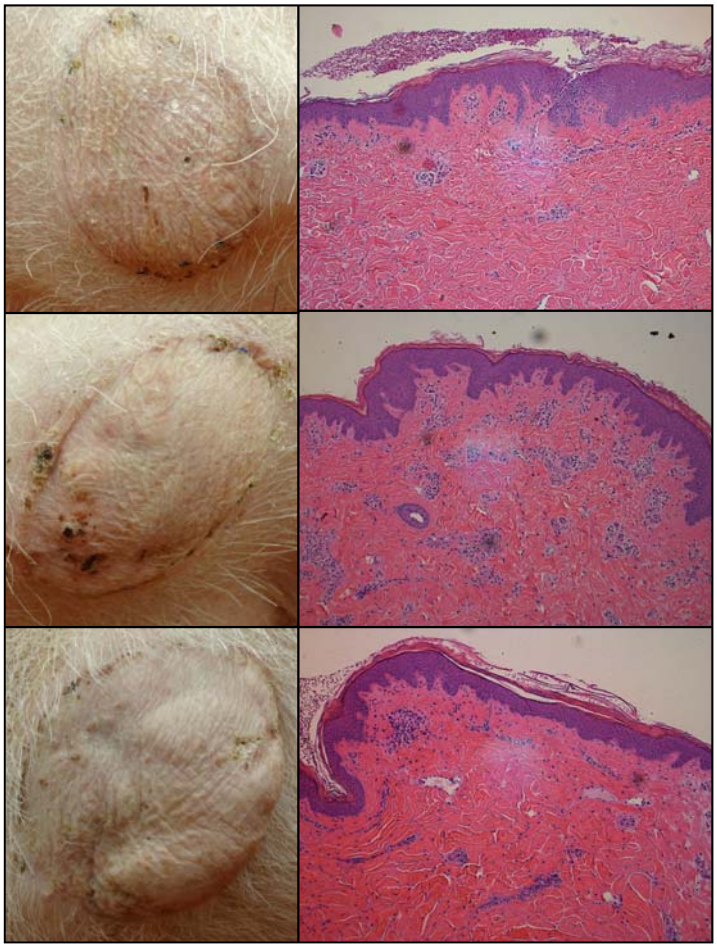

Figure 2. Representative clinical pictures and histological sections stained with hematoxylin and eosin from postoperative day 60 graft skin demonstrating no signs of rejection.

Although all grafts have been maintained without signs of rejection in this high-dose Group, 2 of 3 animals have died prematurely due to complications associated with tacrolimus. Final histopathological diagnosis is still pending for both animals at the time of this report. However, preliminary diagnoses point to infectious complications related to high dose immunosuppression. Pig 22312 experienced sudden respiratory arrest and was found to have an airway obstructing hyperplastic pharyngeal lymph node. On necropsy, generalized lymphadenopathy was found throughout the animal, including mediastinal and mesenteric lymph nodes. A neutrophillic and histiocytic predominance in the lymph nodes points more towards infection rather than post-transplant lymphoprolifereative disease (PTLD). The second premature death in this group, Pig 22227, was due to a gastrointestinal bleed, related to either infectious gastroenteritis/colitis or stress ulcer formation. The third animal, Pig 22229, is currently alive at postoperative day 100, with an intact graft without signs of rejection.

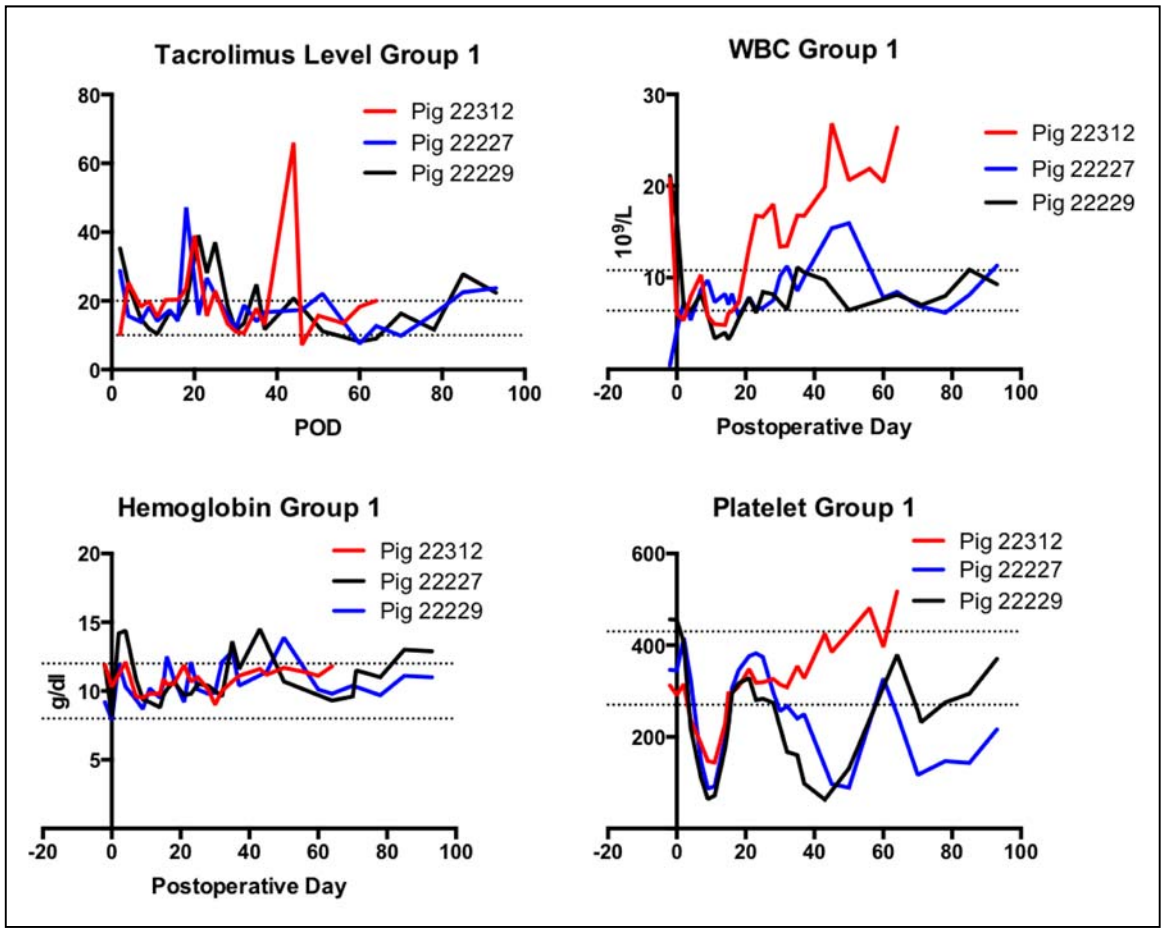

Figure 3. Tacrolimus trough levels, leukocyte count, hemoglobin levels, and platelet counts for Group 1 animals with target or normal ranges outlined by dotted gray lines

Overall, Group 1 has demonstrated viable heterotopic hind limb allografts while receiving high dose tacrolimus. However, 2 out of 3 animals have died prematurely due to complications associated with significant immunosuppression. These findings support our hypothesis that high dose tacrolimus can maintain vascularized composite allotransplants, but does so at the cost of significant complications. Further *in vitro* testing will commence upon completion of all animals in Group 1 (POD 150).

1.3.2 Aim 1, Task 3, Subtask 2: Perform hind limb transplantation with sub-therapeutic, low-dose TAC treatment (Group II; n=3)

One animal, Pig 22309, has been completed in Group 2 low dose tacrolimus (4-6 ng/mL). Upon lowering the dose of tacrolimus to the target range of 4-6 ng/mL, the animal briskly rejected the graft within 20 days, shown in the graft survival curves in Figure 1, the clinical and histological images in Figure 4, and laboratory data in Figure 5. As the graft skin progressed clinically from grade 1 to grade 4 rejection, expected histological changes were encountered: from mild perivascular infiltrates to complete epidermal necrosis with dense diffuse dermal infiltrates. Interestingly, there were only mild

infiltrates in the graft muscle on the day of euthanasia, confirming that the skin component of VCAs rejects first due to high antigenic burden (Figure 6).

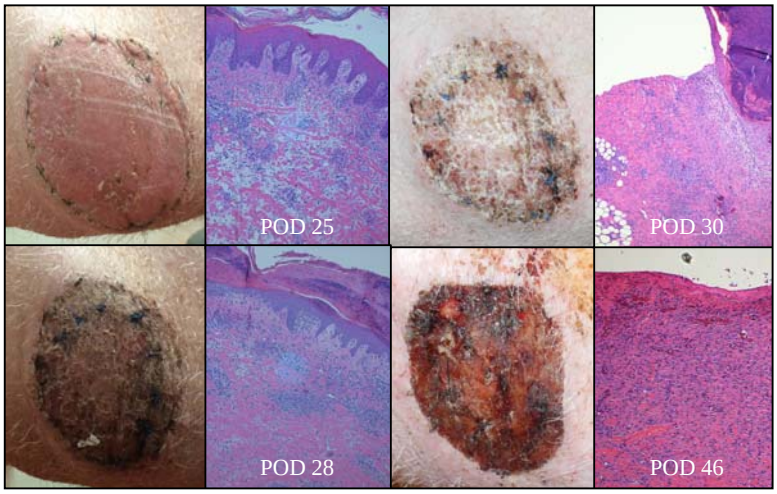

Figure 4. Clinical images (left) with corresponding hematoxylin and eosin stained histological slide (right) of allotransplant graft skin progressing through graded rejection (*POD=postoperative day)

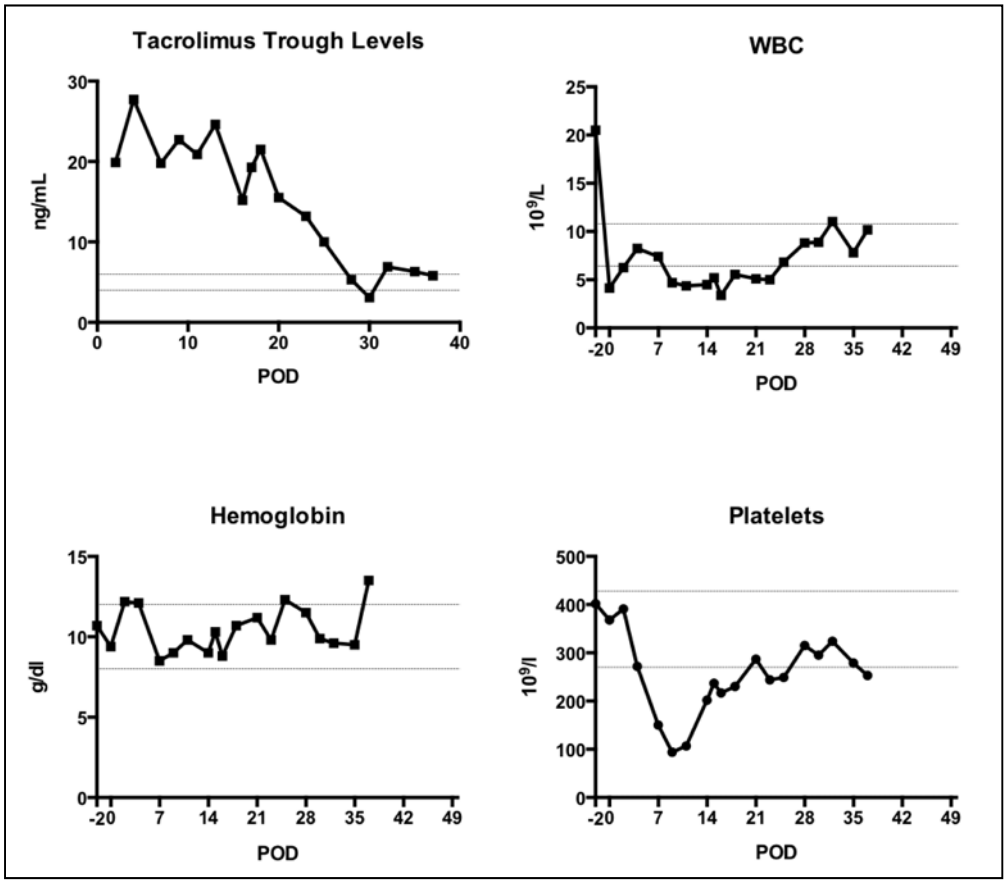

Figure 5. Tacrolimus trough levels, leukocyte count, hemoglobin level, and platelet counts for Group 2 with target or normal ranges outlined by dotted gray lines

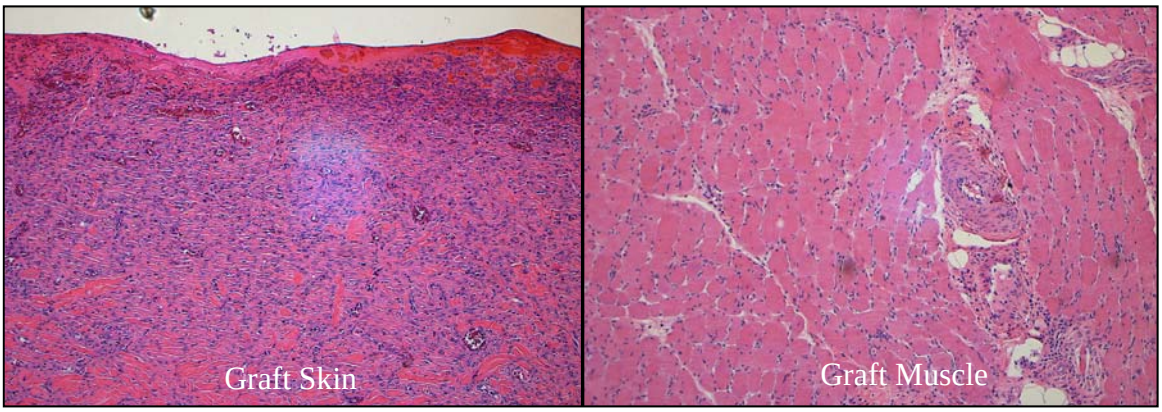

Figure 6. Postoperative day 46 (day of euthanasia) graft skin (left) showing heavy dermal infiltrate and epidermal necrosis and graft muscle (right) showing minimal infiltrate

The first animal from Group 2 demonstrated the inability of low dose tacrolimus (4-6 ng/mL) to maintain the allotransplant and prevent rejection. The remaining 2 animals from Group 2 have yet to be transplanted for reasons that will be detailed in Section 5 – Changes/Problems. *In vitro* studies will commence once all animals from Group 2 have been completed.

1.4 Aim 1, Task 4: Determine impact of peritransplant belatacept treatment to allow for allograft survival with low-dose (sub-therapeutic) CNI treatment

1.4.1 Aim 1, Task 4, Subtask 1: Perform hind limb transplantation with low-dose TAC in combination with belatacept (Group III; n=5)

No animals from Group 3 have been transplanted due to complications with irradiation that will be detailed in Section 5 – Changes/Problems. Prior to continuing with Group 3 animals, which will be receiving belatacept, we want to ensure accurate dosing and optimal/consistent results.

c. What opportunities for training and professional development has the project provided?

All co-investigators involved in the project have received training in *in vivo* and *in vitro* aspects related to the study. Performing the heterotopic hind limb allotransplants is a complex endeavor requiring expertise in surgical principles, microvascular surgery, and transplant surgery. Preoperative planning and coordination is paramount to success, as well as diligent postoperative care of the animals. Moreover, all co-investigators are gaining knowledge and abilities to manage a complex translational large animal project under supervision of the PI.

Professional development is provided during weekly project updates and laboratory meetings, requiring careful preparation of weekly activities and future plans. Preparation for presentations requires individual study and study groups in order to communicate the

details and rationale of the project to other laboratory members. Attendance at conferences and seminars is planned to further develop members of the project.

d. **How were the results disseminated to communities of interest?**

Nothing to report.

e. **What do you plan to do during the next reporting period to accomplish the goals?**

In the next reporting period we plan to finish the remaining transplants for Groups 2 and 3 as well as begin and complete Groups 4 and 5 of Aim 2 (Year 2). We currently have all animals planned for transplantation to finish Aim 1, with full SLA mismatch, selected and housed in our animal facility at Johns Hopkins. Now that concerns regarding irradiation dosing have been resolved (which will be detailed in Section 5), we are prepared to proceed with transplantation of the remaining Aim 1 animals. Following completion of these transplants within the following month, we will be able to continue with transplantation of Aim 2 animals. Although we had a minor set back due to irradiation, surgical technique and postoperative care of the animals is finely tuned at this point, and should make for efficient completion of the remaining groups.

We also plan to complete all *in vitro* testing from Aim 1 animals early in Year 2 following completion of each group. Our laboratory is well versed in the *in vitro* techniques required for these analyses, and this should not create any delays.

4. IMPACT:

a. What was the impact on the development of the principal discipline(s) of the project?

Nothing to report

b. What was the impact on other disciplines?

Nothing to report

c. What was the impact on technology transfer?

Nothing to report

d. What was the impact on society beyond science and technology?

Nothing to report

5. CHANGES/PROBLEMS:

a. Changes in approach and reasons for change

There have been some changes in our approach to the preoperative irradiation procedure that will be detailed below in 5b. Briefly, we now perform a whole body computed tomography (CT) scan of each pig immediately prior to irradiation for precise dose planning.

b. Actual or anticipated problems or delays and actions or plans to resolve them

There have been delays in the progress of the study due to unanticipated complications associated with preoperative irradiation. Preoperative irradiation is performed 2 days prior to transplantation as a means of induction and lymphocyte depletion, similar to our clinical regimen using alemtuzumab for lymphocyte depletion. In the first set of animals transplanted, the whole body (100 cGy TBI) and thymic (700 cGy) irradiation doses, however, were twice the planned dosage due to technical reasons. These animals went on to receive their transplantation, but unfortunately died by postoperative days 14 and 15 due to pancytopenia, and thrombocytopenia in particular, shown in Figure 7. Despite successful surgical transplantation, the animals could not be supported to survive the bone marrow recovery period. Following this incident, our group met with the radiation oncology team who is performing the irradiation procedures to discuss target doses. To ensure our target dose was safe prior to performing another transplantation, two pigs were irradiated with the correct target doses (100 cGy TBI and 700 cGy thymic) and allowed to recover without performing the transplantation. Appropriate bone marrow recovery was achieved (Figure 8) and we felt comfortable moving forward with further transplants.

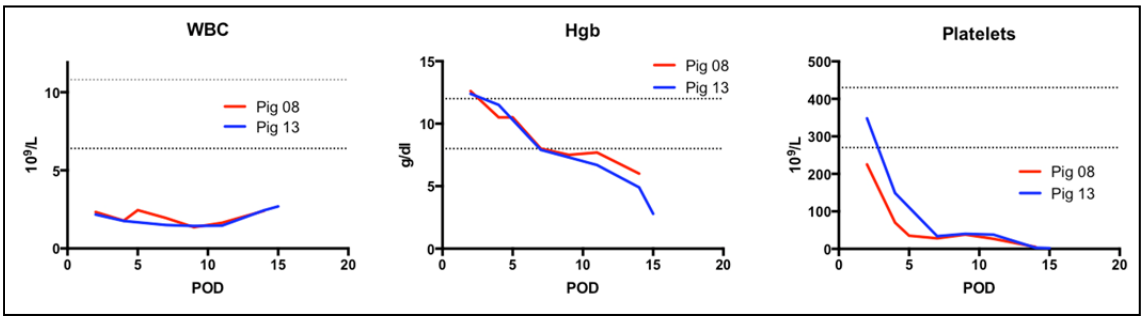

Figure 7. Leukocyte, hemoglobin, and platelet trends following transplantation in animals receiving twice the targeted dose of irradiation

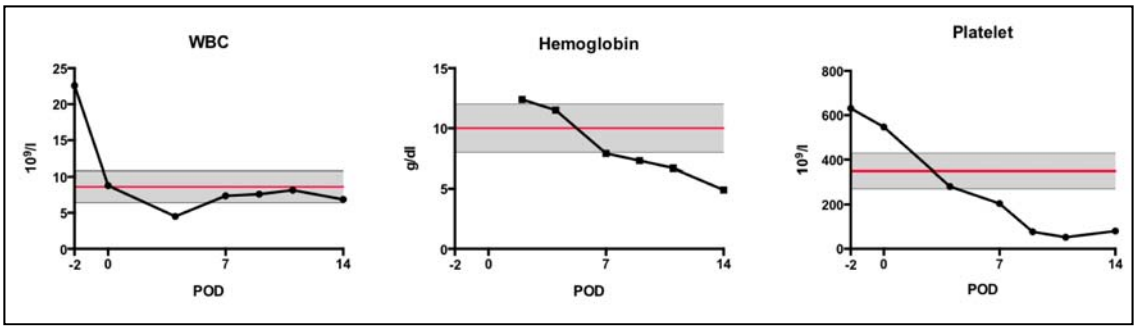

Figure 8. Complete blood count recovery trends following irradiation dose validation. Leukocyte counts have recovered to normal and platelet levels are beginning to show uptrend.

The next two sets of transplants (4 animals) were successful with appropriate bone marrow recovery, and are the animals detailed in the Section 3 within Groups 1 and 2. However, the following set of transplants has shown again problems with preoperative irradiation. One animal died due to sedation and intubation required for irradiation. The second animal underwent irradiation and transplantation, but was found to be pancytopenic by postoperative day 11 (Figure 9), with hemoglobin of 5 g/dl and platelets of 13 K/mm^3. Upon investigation and further discussion with the radiation oncology team, it was calculated that the animal received an approximately 25-30% higher dose than target levels. The animals in this round of transplantation were significantly smaller than previous animals (15 kg versus 35 kg), which significantly affected the irradiation planning, despite weighing and measuring the animals prior to irradiation. To more precisely plan irradiation, a naïve pig underwent whole body CT scan to elucidate the relationship and proportions of the spine to the surrounding tissue, as well as the exact location of the thymus (Figure 10). Using the measurements, the radiation oncology team confirmed that the previous animal received a 29% higher irradiation dose than the target level.

With the above information, we have action plans established and corrective measures in place for the upcoming transplantations. From the groups discussion with the radiation oncology team and performing the whole body CT scan, we are now planning on scanning each animal immediately prior to irradiation in order to precisely calculate the dose and area of irradiation. This will eliminate inaccuracies related to varied animal sizes. We are also decreasing the dose rate of the irradiation, similar to settings used in pediatric patients, as the animals are similar in age (3-6 months old) and size (15-35 kg) to human pediatric patients. Furthermore, we are planning to transport animals awake in rolling cages and only briefly sedate them with ketamine 20 mg/kg and xylazine 2 mg/kg (approved in original protocols) for the short period of time (~30 minutes) required for CT scan and irradiation. This should limit complications related to sedation, as the pigs were previously sedated, maintained on a propofol infusion, and intubated for approximately 3 hours during the entire transport and irradiation procedure.

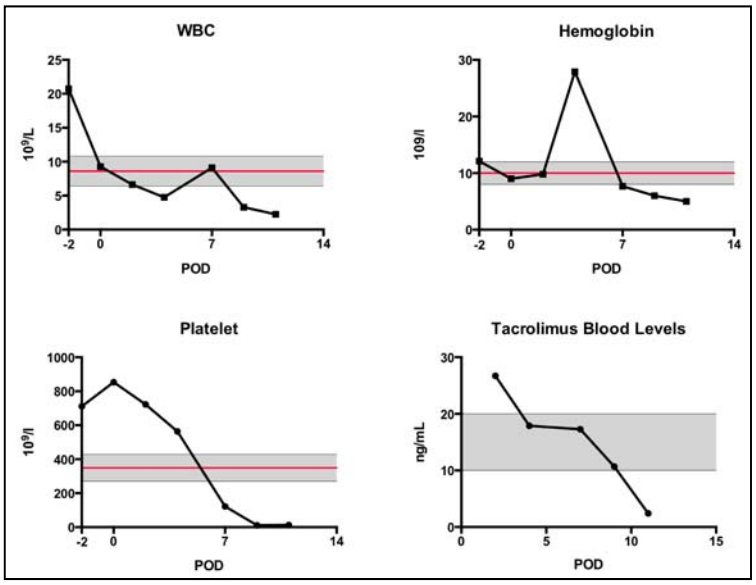

Figure 9. . Complete blood count trends and tacrolimus blood levels in most recent animal receiving higher than targeted irradiation doses showing severe thrombocytopenia

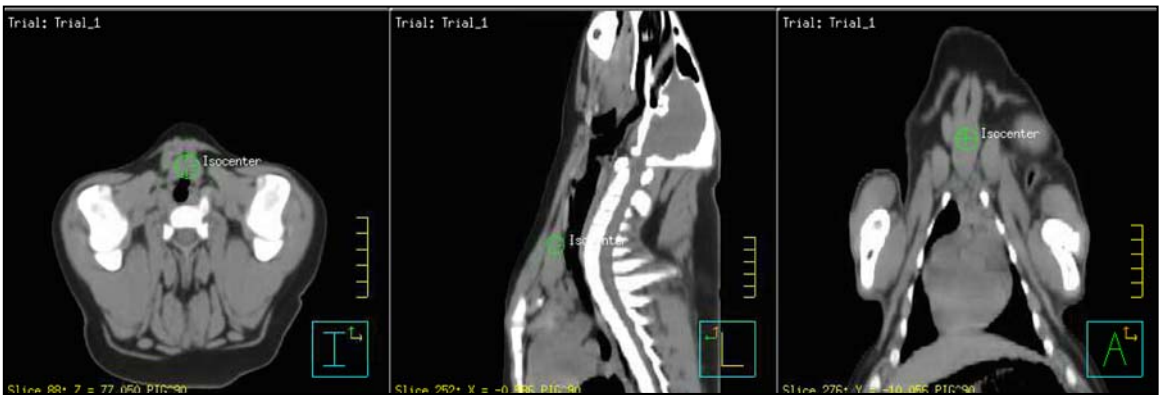

Figure 10. CT scan for planned isocenter for preoperative thymic irradiation

c. Changes that had a significant impact on expenditures

The delays and problems related to irradiation above have caused a significant impact on expenditures. As the transplantation schedule was delayed while irradiation concerns were addressed, less animals were transplanted resulting in less spending on postoperative recovery, medication (belatacept in particular), and *in vitro* analysis (i.e. antibodies, assays, etc.). Furthermore, less animals were being ordered as we were awaiting resolution of irradiation problems, and therefore animal housing expenditures decreased. Now that irradiation problems have been resolved, all animals for Aim 1 have been ordered and are in possession, and housing costs are going to increase significantly. As transplantations are ready to resume at an accelerated pace, expenditures from postoperative care will also increase significantly. Moreover, the problems above have

resulted in animal loss that will impact the overall budget, and once all groups are finished, it will be important for all funds to be available.

d. **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

Nothing to report

e. **Significant changes in use or care of human subjects**

Nothing to report

f. **Significant changes in use or care of vertebrate animals.**

Nothing to report

g. **Significant changes in use of biohazards and/or select agents**

Nothing to report

6. PRODUCTS

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

a. What individuals have worked on the project?

Name: Gerald Brandacher
Project Role: Principal Investigator
Nearest Person Month Worked: 10%
Contribution to Project: Dr. Brandacher oversees all aspects of project planning, execution and data analysis. He actively participated in all animal surgeries.
Funding Support: Grant

Name: W. P. Andrew Lee
Project Role: Co-Investigator
Nearest Person Month Worked: 2%
Contribution to Project: Dr. Lee participated in project planning and data analysis.
Funding Support: Departmental Sources

Name: Justin Sacks
Project Role: Co-Investigator
Nearest Person Month Worked: 5%
Contribution to Project: Dr. Sacks participated in all donor surgeries.
Funding Support: Departmental Sources

Name: Jaimie Shores
Project Role: Co-Investigator
Nearest Person Month Worked: 5%
Contribution to Project: Dr. Shores participated in all recipient surgeries.
Funding Support: Departmental Sources

Name: Damon Cooney
Project Role: Co-Investigator
Nearest Person Month Worked: 5%
Contribution to Project: Dr. Cooney participated in all recipient surgeries, post-transplant care and data analysis.
Funding Support: Departmental Sources

Name: Ned Swanson
Project Role: Post-Doctoral Fellow
Nearest Person Month Worked: 50%
Contribution to Project: Dr. Swanson participated in all recipient surgeries, pre and post-transplant care, performed *in vitro* assays and data analysis.
Funding Support: Grant

Name: BC Oh
Project Role: Lab Technician
Nearest Person Month Worked: 25%
Contribution to Project: Dr. Oh participated in all recipient surgeries, pre and post-transplant care, performed *in vitro* assays and data analysis.
Funding Support: Grant

b. Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

i. Nothing to Report

c. Partnering Organization

i. Organization Name: Massachusetts General Hospital
ii. Location of Organization: Boston, MA

Massachusetts General Hospital provided support and consultation with regard to donor/recipient selection and matching as well as post-transplant immunological *in vitro* assays.

d. SPECIAL REPORTING REQUIREMENTS

a. **QUAD CHARTS:** Attached.

e. APPENDICES

Nothing to report.

Immunomodulation and Tolerance Induction after VCA Using Biologic Agents (CTLA4-Ig) and Donor Bone Marrow Cells

MR120034P10, Restorative Transplantation Research
Award Number: W81XWH-13-2-0060

PI: Gerald Brandacher, M.D. **Org:** Johns Hopkins University School of Medicine **Award Amount:** $1,297,034

Study/Product Aim(s)

- Establish a belatacept-based protocol to enable CNI minimization after Vascularized Composite Allotransplantation (VCA).
- Investigate the possibility to convert from conventional CNI-based immunosuppression to belatacept maintenance with subsequent CNI withdrawal.
- Compare immunomodulatory donor bone marrow (BM) infusion (BMI) to BM transplantation (BMT) with establishment of durable mixed chimerism for induction of tolerance and/or VCA survival on CNI-free immunosuppression using a belatacept-based regimen.

Approach

In this study we propose to develop novel protocols using donor bone marrow cells and FDA-approved biologic agents (Cytotoxic T-lymphocyte-associated antigen-4 immunoglobulin [CTLA4-Ig], belatacept) for the induction of immune tolerance with minimal or only transient immunosuppression in de-novo VCA recipients and to allow withdrawal of calcineurin inhibitors (CNIs) from patients that have already been transplanted under conventional immunosuppression. Studies will be performed using a translational large animal model for VCA as outlined in Figure 1.

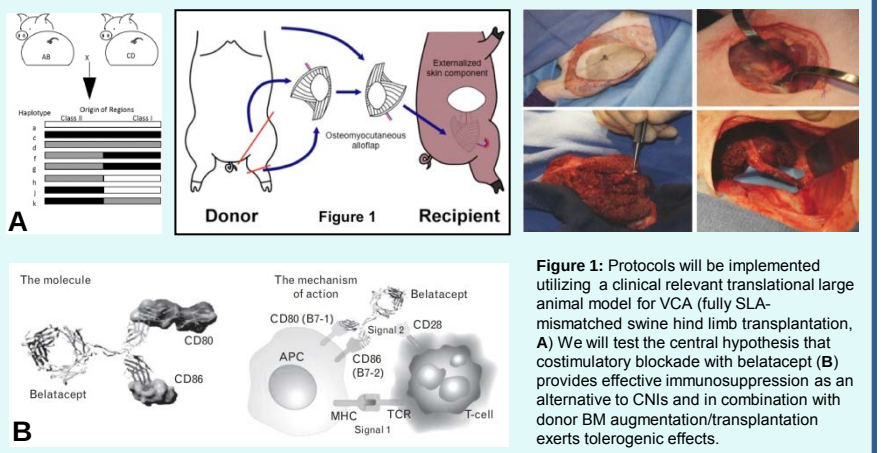

Figure 1: Protocols will be implemented utilizing a clinical relevant translational large animal model for VCA (fully SLA-mismatched swine hind limb transplantation, **A**) We will test the central hypothesis that costimulatory blockade with belatacept (**B**) provides effective immunosuppression as an alternative to CNIs and in combination with donor BM augmentation/transplantation exerts tolerogenic effects.

Accomplishment: Implemented successfully first clinical protocol for upper extremity transplantation using donor bone marrow cell therapies and tacrolimus monotherapy.

Timeline and Cost

Activities	CY 14	15	16
Establish a belatacept-based protocol to enable CNI minimization after VCA (JHU)			
Test ability to convert from conventional CNI-based immunosuppression to belatacept maintenance with subsequent CNI withdrawal (JHU)			
Use BMI combined with CNI-free belatacept-based regimen to establish chimerism and tolerance induction after VCA (JHU)			
Use BMT combined with CNI-free belatacept-based regimen to establish chimerism and tolerance induction after VCA (MGH)			
Estimated Budget ($K)	**$379,907**	**$366,875**	**$633,096**

Updated: (10/13/2014)

Goals/Milestones

CY14 Goals – *CNI minimization*

- ✓ Adapt clinically established induction and CNI maintenance regimen in this translational large animal VCA model
- ☐ Determine impact of peri-transplant belatacept treatment to allow for allograft survival with low-dose CNI treatment

CY15 Goals – *CNI withdrawal*

- ☐ Attempt CNI weaning/withdrawal without CTLA4-Ig and assess time course of allograft rejection
- ☐ Perform CNI weaning/withdrawal with delayed belatacept treatment and maintenance

CY16 Goals – *CNI free immunosuppression and tolerance induction*

- ☐ Determine impact of BMI vs. BMT combined with short-term CNI on immunomodulation and allograft survival
- ☐ Develop tolerance protocol combining optimized BM regimen with short-course CNI and peri-transplant belatacept treatment
- ☐ Develop tolerance protocol combining optimized BM regimen with short-term CNI, peri-transplant and short course post-transplant belatacept treatment

Comments/Challenges/Issues/Concerns

- Experiments as outlined by SOW are in progress, validation and adaptation of induction regimen completed

Budget Expenditure to Date

Projected Expenditure: NA
Actual Expenditure: $186,944.57